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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Weiner, David B. *et al.* #14

Serial No.: 08/453,349

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Group Art Unit for Parent Application: 1806

Examiner for Parent Application: J. Railey

For: **Genetic Immunization**

Assistant Commissioner of
Patents
Washington, D.C. 20231

Dear Sir:

DECLARATION OF DAVID B. WEINER UNDER 37 C.F.R. § 1.132

I, David B. Weiner do hereby declare that:

1. I am an Associate Professor of Pathology and Laboratory Medicine at the University of Pennsylvania School of Medicine in Philadelphia, Pennsylvania. Attached hereto as Exhibit A is a copy of my Curriculum Vitae.

2. I am a co-inventor of the subject matter claimed in the above-identified patent application.

3. I have participated in the design and/or supervised the performance of experiments to determine whether genetic immunization as claimed in the application can be used to protect individuals from pathogen infection and to treat individuals infected by pathogens.

4. Included in such experiments are experiments to test whether genetic immunization can be used to protect individuals from HIV-1 infection and to treat individuals who are infected with HIV-1. These experiments include the following:

a) Experiments using a SHIV model in which cynomolgus macaque monkeys were vaccinated against HIV-1 and subsequently challenged with HIV-1/SIV hybrid virus;

b) Experiments using HIV-1-infected chimpanzees to determine the therapeutic effect of an HIV-1 vaccine of the invention; and

c) Human clinical trials testing the therapeutic effect of genetic immunization in human patients infected with HIV-1.

5. The experiments using the SHIV model are described in paragraphs 6 to 14 below. The experiments using the chimpanzee model are described in paragraphs 15 to 22 below. The human clinical trials are described in paragraph 23 below.

6. The SHIV model is described in Li, J. *et al.* 1992 *J. Acq. Immun. Defic. Syndr.* 5(7):639-646, which is attached hereto as Exhibit B. According to this model for HIV infection, a hybrid SIV/HIV virus is provided which can replicate at high levels in cynomolgus monkeys and express HIV-1 envelope glycoproteins. HIV-1 vaccines which use envelope glycoproteins as target antigens can be tested to determine if they protect monkeys from infection by the SHIV hybrid virus.

7. Experiments using the SHIV model were performed. A total of six (6) cynomolgus monkeys was used including two (2) control animals, and four (4) test animals vaccinated with gene constructs that encode HIV-1 envelope proteins.

a) One control was a naive animal that received no treatment.

b) The other control was vaccinated with bupivacaine and vector DNA that did not have HIV-1 coding sequences.

c) Vaccinated animals 4, 5 and 6 were treated with bupivacaine prior to vaccination with gene constructs. Test animals that were administered bupivacaine were vaccinated with gene constructs that contained coding sequences for the envelope protein from HIV-1 strain Z6 in phosphate-buffered saline 24 hours after bupivacaine administration. Vaccinated animal 3 was

vaccinated with the same gene construct formulated with bupivacaine. The vaccinated animals were subsequently boosted two times with gene constructs that contained coding sequences for the envelope protein from HIV-1 strain MN in phosphate buffered saline. One boost vaccine administration was performed following bupivacaine administration given 24 hours prior to boost. The vaccination protocol is summarized in Table 1, which is attached hereto as Exhibit C.

8. Serology studies were performed before control and vaccinated animals were challenged to determine whether the animals had antibodies to HIV-1 proteins encoded by envelope genes from specific HIV-1 strains. The results of pre-challenge serology studies are shown on Table 2, which is attached hereto as Exhibit D.

9. The animals were subsequently challenged with a SHIV virus construct. The virus construct, SHIV IIIB, is a chimeric SIV/HIV-1 virus containing HIV-1 strain IIIB envelope glycoprotein. The SHIV IIIB construct was provided by Virus Research Institute who reported that it contained 4600 $TCID_{50}$/ml as titered in CEMx174 cells. The SHIV IIIB construct was transfected in CEMx174 cells and grown in rhesus monkey PMBCs to provide the cell-free virus

stock. Each animal was challenged with a dose of 50 animal infectious doses (AID) of SHIV IIIB in 1 ml delivered intravenously. One AID was determined to be approximately equal to 1 $TCID_{50}$ as follows. The virus stock was inoculated intravenously into rhesus monkeys to determine the AID via p27 assays on supernatants from serial virus load co-cultures of rhesus PBMCs with CEMx174 cells.

10. After intravenous challenge of animals 1-6 with 50 AID of SHIV IIIB, the extent of infection with the virus was evaluated by periodically collecting whole blood for determination of virus load by limiting dilution co-culture.

11. Both control animals developed acute infection and early viral replication. The levels were slightly lower than expected based upon historical data for rhesus monkey infection with SIVmac251 or SHIV IIIB.

a) Inoculation of a naive cynomolgus monkey (#1) with SHIV IIIB resulted in virus loads (ID_{50} = 2.5-5.5) slightly lower than mean virus loads compared to the historical data for naive rhesus monkeys inoculated with SIVmac251 or SHIV IIIB. Virus load was negligible (ID_{50} < 1.0) by Day 63 in this animal.

b) The one cynomolgus monkey (#2) vaccinated with control (non-HIV) vaccines only had virus loads (ID_{50} = 3.5-7.0) similar to mean virus loads for rhesus monkeys challenged with SIVmac251 or SHIV IIIB; these were likewise negligible by Day 63.

12. Three of the four vaccinated animals controlled early viral replication; one did not. One of three animals which controlled early viral replication was considered protected.

a) One animal (#4) developed relatively high virus loads (ID_{50} = 5.5 to > 12.0) and retained a moderate virus load through Day 63 (ID = 3.0).

b) The two other animals (#5 and #6) had low virus loads (ID_{50} = 1.5 and 2.0) on Day 8. The virus load levels in animals #5 and #6 was observed to be lower at an earlier date than the date when any other animals were observed to have similarly low viral load levels. Both of animals #5 and #6 developed modest virus loads (ID_{50} = 1.5-4.5) and appeared to clear the virus by Day 63.

c) The fourth animal vaccinated against HIV (#3) is considered to be protected against early replication of SHIV IIIB. This animal had negligible virus loads (ID_{50} < 1.0) on all days except Day 15, when virus load was very low (ID_{50} = 1.5). This animal had been vaccinated using the injection gun, supplemented

with intramuscular injections of the test vaccine, with or without pretreatment by injection of bupivacaine.

d) Figure 1, which is attached hereto as Exhibit E, shows the Average viral load during SHIV challenge as determined in PBMC collected on a weekly basis following the viral challenge. Virus load was determined by limiting dilution in viral culture. Panel A shows an average of two control animals and of four vaccinated animals. Panel B shows results from the two control animals. Panel C shows results from animal 3 which was immunized with test vaccine.

13. Each of the three vaccinated animals that controlled early viral replication demonstrated no seroconversion to the SHIV IIIB envelope glycoprotein following challenge. The one vaccinated animal that did not control early viral replication showed seroconversion to the SHIV IIIB envelope glycoprotein following challenge. Data from studies of serology following SHIV challenge and virus load is shown in Figure 2, which is attached hereto as Exhibit F. Cytolytic T cell responses were observed in both the protected vaccinated animal and one of the other vaccinated animals that controlled early viral replication. Data from studies of pre-challenge cytotoxic T lymphocyte activity is shown in Figure 3, which is attached hereto as Exhibit G.

14. In summary, inoculation of cynomolgus monkeys not previously vaccinated against HIV-1 with SHIV IIIB virus resulted in a burst of virus replication, as expected. Virus loads were slightly lower than in rhesus monkeys challenged with SIVmac251 or SHIV IIIB, perhaps due to differences in animal species or challenge dose. Three of four cynomolgus monkeys previously vaccinated against HIV-1 via intramuscular injection of the test vaccines did not seroconvert when challenged. Two of these three animals controlled early viral replication after challenge with SHIV IIIB and the third animal was protected against early SHIV IIIB replication.

15. The chimpanzee as a model for HIV is described in:

a) Neurath, A.R. *et al.* 1991 *AIDS Res. Hum. Retroviruses* 7:813, which is attached hereto as Exhibit H;

b) Berman, P.W. *et al.* 1994 *AIDS* 8:591-601, which is attached hereto as Exhibit I;

c) Emini, E. *et al.* 1990 *J. Virol.* 64:3674-3678, which is attached hereto as Exhibit J; and

d) Schultz, A.M. and S-L. Hu 1993 *AIDS* 7:S161-S170, which is attached hereto as Exhibit K.

16. The following vaccination protocol was used in experiments that were done with the chimpanzee model:

a) Each dose of the test vaccine and the control vaccine contained 100 μg DNA in 0.5 ml 0.25% bupivacaine. The DNA in the test vaccine was an expression vector that included the coding sequence of HIV-1 envelope protein (gp160), the rev gene, a partial nef gene and a partial vpu gene from HIV-1 strain MN. The DNA in the control vaccine was the expression vector used in the test vaccine without the inclusion of the HIV-1 sequences.

b) Two adult male chimpanzees (Pan troglodytes) were used in the study. Both chimpanzees were infected with HIV-1 IIIB; antibody against p24 was detected in the serum of both animals which were infected for at least three months before assignment to the study. The two chimpanzees were acclimated to laboratory conditions for at least 4 weeks prior to treatment.

c) Animals were randomly selected to be the test chimpanzee (CHIMP #1) and control chimpanzee (CHIMP #2). The test vaccine was directly injected into muscle tissue of one HIV-1 infected chimpanzee (CHIMP #1) whereas the other HIV-1 infected chimpanzee (CHIMP #2) received the control vaccine.

d) The test vaccine and control vaccine was administered to CHIMP #1 and CHIMP #2, respectively, by intramuscular needle injection into the triceps muscles. A total of 3 doses are administered at 6 week intervals.

17. The effects of the test vaccine and control vaccine on the general health of the chimpanzees were assessed and the results of this study suggest the safety of both the test vaccine and control vaccine on injected animals. With regard to the effect of the test vaccine on the general health of the animals, there were no significant changes between CHIMP #1 (test chimpanzee) and CHIMP #2 (control chimpanzee) in any of the study parameters tested. This is seemingly a strong indication of the safety of the test vaccine.

18. The efficacy of the test vaccine was assessed. This study was developed and conducted to determine if immune responses specific to HIV-1 proteins could be altered in HIV-1 infected chimpanzees after intramuscular administration of DNA expression plasmid that included HIV-1 coding sequences.

19. Viral load was measured in each chimpanzee and compared by co-culture procedure and reverse transcriptase assay (RT). The assay was performed by pelleting the virus from the culture supernatant and disrupting the virus to release the RT enzyme. The enzyme reacts with the cocktail mixture that includes an RT substrate and nucleotides including [^{3}H] thymidine. Incorporation of [^{3}H] thymidine was measured as an indication of RT activity. The results of RT assay are presented in Table 3, which is attached hereto as Exhibit L. The control animal, CHIMP #2, had a high RT activity on study week 17 and 25. This is a normal variation consistent with HIV-1 infection in chimpanzees.

20. Measurement of HIV-1 viral burden was also done by HIV-PCR assay. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation. Isolated PBMCs were frozen and stored in liquid nitrogen until use. HIV-PCR was performed using primers to amplify fragments of DNA from the gag region of the HIV-1 genome. The products of amplification were analyzed by 5% agarose gel electrophoresis and stained with ethidium bromide. HIV-1 DNA was detected in the amplified products of samples from all bleeding times. The results of amplification of DNA are shown in Table 4, which is attached hereto as Exhibit M. Figure 4, which

is attached hereto as Exhibit N, is a copy of the photograph of the ethidium bromide stained electrophoresis gel.

21. With regard to the effect of the test vaccine on the HIV infection, the results indicate a positive effect. A weaker antibody response against gp41 was detected in the treated animal than in the control animal as determined by Western blot analysis. By reverse transcriptase assay and polymerase chain reaction analysis, the treated animal appeared to have a much lower virus load than the control animal. The change in the viral load was more obvious as study progressed through week 25. The data listed in Table 4 and shown on Figure 3 indicate that the viral load is decreased in the vaccinated animal after week 15.

22. The results of this study suggest that the immunotherapy regimen of the test vaccine showed some efficacy as an immunotherapeutic for controlling HIV-1 infection.

23. An IND was approved by the Food and Drug Administration for testing vaccines of the invention in Human clinical trials. Clinical trials have been initiated at the Hospital of the University of Pennsylvania with the test vaccine in human patients.

24. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like a punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the applications or any patent issued thereon.

DATE: 8/25/95

DAVID B. WEINER

Attachments:

Exhibit A - Curriculum Vitae of David B. Weiner
Exhibit B - Li, J. *et al.* 1992 *J. Acq. Immun. Defic. Syndr.* 5(7):639-646
Exhibit C - Table 1
Exhibit D - Table 2
Exhibit E - Figure 1
Exhibit F - Figure 2
Exhibit G - Figure 3
Exhibit H - Neurath, A.R. *et al.* 1991 *AIDS Res. Hum. Retroviruses* 7:813
Exhibit I - Berman, P.W. *et al.* 1994 *AIDS* 8:591-601
Exhibit J - Emini, E. *et al.* 1990 *J. Virol.* 64:3674-3678
Exhibit K - Schultz, A.M. and S-L. Hu 1993 *AIDS* 7:S161-S170
Exhibit L - Table 3
Exhibit M - Table 4
Exhibit N - Figure 4